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Pre- and Posttranslational Regulation of Neurosecretion

There has been a remarkable progress in the understanding of the basic mechanism of neurosecretion during the last decade. Little is known, however, about how cells can regulate through different isoforms (pretranslational regulation) and phosphorylation (posttranslational regulation) of exocytotic proteins, which is the basis for neuronal adaptation and synaptic plasticity. One of the key players of neurotransmitter release in both neurons and neuroendocrine cells is the neuronal SNARE complex consisting of synaptobrevin/VAMP, syntaxin 1 and SNAP-25. Several isoforms and phosphorylation sites of the SNARE proteins have been identified, but their role in the regulation of transmitter release is still to be understood.

I studied the role of SNAP-25 splice variants and its close non-neuronal relative SNAP-23 in exocytosis from adrenal chromaffin cells secreting catecholamines from large dense-core vesicles (LDCVs). In separate studies I also investigated the role of the two described phosphorylation sites of SANP-25 in the regulation of cathecolamine release. For evaluation of LDCV exocytosis I used high time resolution patch-clamp capacitance and amperometric measurements together with Ca^{2+} measurements with a combination of pharmacological and genetic techniques. I also developed a new preparation, culturing adrenal chromaffin cells from single embryonic mice that allows us to combine knockout with overexpression techniques, which further increases the sensitivity and specificity of our approach to study neurosecretion.

The findings I present in my Ph.D. Thesis can be summarized in the following five points.

(A) The absence of endogenously expressed SANP-25 leads to the complete absence of an exocytotic burst (fast, synchronized catecholamine release) in chromaffin cells obtained from SNAP-25 knockout mice. However, slow Ca^{2+}-dependent release still remained with almost unchanged single fusion characteristics. The defect in mutant chromaffin cells was due to impaired priming, whereas morphological docking (measures by electron microscopy) remained normal. Acute viral overexpression of SNAP-25a (the predominant isoform in chromaffin cells) reconstituted normal secretion. Thus the observed knockout phenotype is due to the lack of SNAP-25.

(B) Overexpression of SNAP-25 in knockout cells increased secretion, but the fast, burst like component of secretion, characteristic to normal cells, could not be reconstituted. Moreover, overexpression of SNAP-23 led to a decrease of secretion in control cells indicating that SNAP-23 can replace SNAP-25 in the SNARE complex, which is necessary for fast neurotransmission.

(C) The splice variant SNAP-25b resulted in 2-3 fold larger release-ready vesicle pools (estimated from the burst size) than SNAP-25a without changing the release rates. The slow sustained component of secretion that serves for measuring priming rate in the chromaffin system was also unaffected. According to our current model, priming of LDCVs is reversible in chromaffin cells, and the number of primed vesicles is defined by the opposing effect of priming and *de*-priming. Thus SNAP-25b apparently reduces the depriming rate, therefore stabilizing primed vesicles in the release-ready state.

(D) In a separate study I observed that overexpression of point mutations of SNAP-25 at the protein kinase A (PKA) phosphorylation site Thr^{138} led to two distinct phenotypes. T138D mutant, which may mimic the constitutively phosphorylated state (“phosphomimetic”), did not change secretion, whereas T138A (“non-phosphomimetic”) mutant reduced the size of the exocytotic burst. Pharmacological manipulations of PKA activity led to similar changes in secretion: PKA-activation did not change secretion but PKA-inhibition reduced burst size. Moreover, the reduction of the exocytotic burst caused by PKA inhibition could be prevented by inhibition of calcineurin, but not of other phosphatases. Overexpression of the phosphorylation mutants of SNAP-25 could partially rescue PKA-inhibition suggesting that there is at least one other – yet unknown – PKA target involved in the PKA-dependent

regulation of catecholamine secretion. Together these data show that PKA is constitutively active under our experimental conditions and regulates the steady-state size of the releasable pools, partially through phosphorylation of SNAP-25.

(E) In the third (but chronologically the oldest) part of my work I studied the effect of the protein kinase C (PKS) dependent phosphorylation of SNAP-25 at Ser^{187}. I found that PKC – unlike PKA – is only activated after the first stimulation by the elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and is necessary for refilling the releasable vesicle pools emptied by the first stimulation, because inhibition of PKC reduced the exocytotic response to the second but not the first stimulus. Overexpression of the “non-phosphomimetic” S187C mutant had similar effect to PKC-inhibition. However, overexpression of the “phosphomimetic” S187E mutant could not prevent the detrimental effect of PKC-inhibition on the second flash indicating that PKC-dependent phosphorylation of SNAP-25 is necessary but not sufficient for the PKC-dependent potentiation of vesicle recruitment.